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TAMPA BAY AREA
PHOTOCHEMICAL OXIDANT STUDY

AUGUST 1978
FINAL REPORT
APPENDIX C

MONOTERPENE EMISSION RATES
FROM SLASH PINE

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Monoterpene Emission Rates from Slash Pine

by

David T. Tingey, Marybeth Manning, Hilman C. Ratsch

Walter F. Burns, Louis C. Grothaus, and Richard W. Field

U.S. Environmental Protection Agency

and

Northrop Services Incorporated

200 S.W. 35th Street

Corvallis, Oregon 97330

ABSTRACT

There is a growing awareness concerning the role of vegetation as a source of reactive hydrocarbons that may serve as photochemical oxidant precursors. This study assessed the influence, independently, of light and temperature on monoterpene emissions from slash pine (Pinus elliottii Engelm.). Plants were pre-conditioned in a growth chamber then transferred to an environmentally controlled gas-exchange chamber. After samples of the chamber atmosphere were collected, the monoterpenes were concentrated cryogenically and measured by gas chromatography. Five monoterpenes, α-pinene, β-pinene, myrcene, limonene and β-phellandrene were present in the vapor phase surrounding the plants in sufficient quantity to measure reliably. Light did not directly influence monoterpene emission rates since emissions were similar in the dark and at various light intensities. Monoterpene emission rates increased exponentially with temperature (i.e. emissions depend on temperature in a log-linear manner). The sum of the 5 monoterpenes ranged from 3 to 21 μg C/g dry wt/hr as temperature was increased from 20 to 46° C.

INTRODUCTION

High levels of ozone have been measured in rural and remote locations far from significant anthropogenic sources of oxidant precursors. These elevated levels in rural and remote areas could result from transport and/or photo-oxidation of biogenic hydrocarbons. released in the area. Reports of several researchers (i.e., Rasmussen and Went, 1965; Whitby and Coffey, 1977) indicated that volatile organics, including monoterpenes were detected in the atmosphere, and suggested that they had a biogenic origin. Other studies using encapsulation techniques have shown that plants can emit significant quantities of monoterpenes into the atmosphere (Rasmussen, 1972; Hanover, 1972; Arnts et al., 1978; Tyson et al., 1974). However, only limited data (mainly for α-pinene) are available concerning factors that influence emission rates. Light apparently does not directly influence monoterpene emissions even though photosynthate is required for biosynthesis (Rasmussen, 1972; Dement et al., 1975). The emissions of camphor (Tyson, et al., 1974) and α-pinene increase with temperature (Rasmussen, 1972; Arnts et al., 1978; Kamiyama et al., 1978). However, the data of Rasmussen (1972) were developed in a static chamber and may be in error.

The objectives of this study were to: 1) determine monoterpene emission rates from intact plants under controlled environmental conditions, 2) determine the independent influence of light and temperature on emission rates, and 3) develop a model to predict monoterpene emissions which could be used to adjust emission rates determined in the field to standard conditions.

MATERIALS AND METHODS

PLANT CULTURE. Slash pine (Pinus elliotti Engelm.) plants were obtained from the Division of Forestry, Florida Department of Agriculture and Consumer Services as bare root seedlings and potted in 15-cm pots in a Jiffy mix*; perlite (1:2; V:V) mixture. Plants were cultured in a greenhouse at maximum day/night temperatures of 28° and 20° C, respectively. Sunlight was supplemented and the photoperiod extended to 16 hr per day with light from HID sodium vapor lamps. The plants received 1/2 strength modified Hoagland's nutrient solution daily. At least 4 weeks before sampling, slash pine trees were placed in a growth chamber and conditioned at maximum day/night temperatures of 27° and 18° C, respectively with a 16 hour photoperiod. When samples were taken, the plants had both mature and young elongating needles.

GAS-EXCHANGE SYSTEM. The gas-exchange system used to determine emission rates (Figure 1) consisted of 1) a gas-exchange chamber to enclose the foliage, 2) an air flow system that controlled CO_2 concentration, dewpoint, and provided hydrocarbon free air (pure air source) to the gas-exchange chamber, and 3) a monitoring system that measured CO_2 concentration and dewpoint of the atmosphere entering and exiting the plant chamber, light intensity within the plant chamber and air temperature.

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GAS-EXCHANGE CHAMBER. The gas-exchange chamber was similar to the one described by Huang et al., (1975) with mixing characteristics of a constant stirred tank reactor (Rogers et al., 1977). The gas-exchange chamber consisting of 2 independent chambers for the foliage and pot- root mass, respectively (Figure 2) was designed for use in a controlled environment chamber which regulated light and cooling. In each experiment, the upper chamber was removed to insert the pot-root mass into the lower chamber. The two halves of the 24-cm diameter disk with center cut out were fitted around the stem, set in place and sealed to the stem and chamber with modeling clay. The upper chamber was placed over the plant without injuring needles and sealed to the chamber base. Both upper and lower chambers contained impellers with exterior motors and the upper chamber contained 2.5 cm high baffles arranged equidistantly around the walls of the chamber to insure well mixed air. The heating element and temperature sensor were connected to a Love model 48* temperature controller. Within the upper chamber, air temperature was monitored with a shielded thermocouple and light intensity (400-700 nm) with a Lambda Instruments model LI-190SR Quantum Sensor*. Various light levels were obtained by a step wise increase in the number of incandescent and fluorescent lights in the controlled environment chamber and the addition of a sodium vapor lamp.

AIR-FLOW SYSTEM. Air was pumped through an Aadco* pure air generator to remove hydrocarbons, CO_2, and reduce the dewpoint (Figure 1). Carbon dioxide and water vapor were added back to the air stream to obtain desirable levels. Air flow into the gas-exchange chamber was adjusted by a valve, monitored by a flow meter and ranged from 2 to 5 l/min depending on plant size and environmental conditons. Air samples for CO_2, dewpoint and hydrocarbon analyses were taken from sample ports at the chamber's inlet and outlet, respectively.

Carbon dioxide concentration and dewpoint of the air stream were monitored with an infrared gas analyzer and dewpoint hygrometer, respectively. The dewpoint of the air entering the gas-exchange chamber was held constant during each experimental run. Inlet dewpoints ranged between -6 and 0° C and were obtained by mixing dry air from the pure air generator with humidified air. The dewpoint of air exiting the chamber varied between 26 and 38° C, depending on experimental conditions. Inlet and outlet CO_2 concentrations of the air stream ranged from 400-600 and 310-390 µl/l, respectively.

HYDROCARBON SAMPLING AND ANALYSIS. The monoterpenes were separated on a 15.2 m x 0.5 mm ID stainless steel support coated open tubular (SCOT) column coated with 4% carbowax 20 M (Helium carrier, 4 cm^3/min) and quantified with a flame ionization detector (FID). Since an FID responds linearly to the mass of organic carbon (David, 1974), a 1.01 µl/l isooctane external standard was used to calculate the mass of organic carbon emitted for each terpene. Three to six 1 ml isooctane standards were taken each day with a reproducibility of ± 2%. Standards of each of the monoterpenes were used to determine their retention time.

For each analysis 25 to 50 ml air samples were collected from the sample port of the gas-exchange chamber using a 100 ml pressure LOC* syringe. The samples were injected through a K_2CO_3 filter to remove water and a 6 port valve onto a stainless steel trap (61 cm x 0.25 mm ID) immersed in liquid oxygen to concentrate the hydrocarbon samples (Rasmussen, et al., 1974). Following sample injection, the stainless steel trap remained in the cryogen for a 4 min period with a helium purge flow (13 cm^3/min). The concentrated sample was volatilized onto the column by heating rapidly the trap in boiling

water.

Positive identification of the monoterpene emissions from slash pines were made by a combination of gas chromatography and mass spectrometry. The monoterpene mass spectra were compared to the EPA Mass Spectral Search System (MSSS), Registry of Mass Spectral Data (Stenhagen, et al., 1974) and monoterpene standards to confirm identification.

EXPERIMENTAL DESIGN. The influence of temperature on monoterpene emissions at various light levels was studied by increasing temperature from 20 to 46° C in 4 to 6° increments at each of 4 light levels (approximately 100, 200, 400 or 800 μeinsteins/m^2/sec). To study the effect of light intensity on monoterpene emissions, light intensity was increased in a step wise manner (0, 100, 200, 400 and 800 μeinsteins/ m^2/sec) at each of 4 temperatures (29, 35, 40 or 46° C). After each change of light or temperature, a 60 minute equilibration time was observed before collecting duplicate air samples for hydrocarbon analysis. A minimum of three plants was used to develop each temperature or light response curve. After each experiment, needles were removed from the slash pine and dry weight was measured after the needles dried at 70° C for 72 hours.

Emission rates for each monoterpene (μg C/g dry wt/hr) from slash pine were calculated using the following equation:

$$\text{monoterpene emission rates} = \frac{J\ \Delta conc}{W}$$

J = air flow rate through the gas-exchange chamber (l/hr)

ΔConc = change in monoterpene concentration of air as a result of passage through the gas-exchange chamber (μg/l). There were no monoterpenes in the air entering the chamber.

W = total needle dry weight of the plant (g)

DATA ANALYSIS. The relationship between the means and standard deviations of samples taken at each light and/or temperature point indicated that monoterpene emission rates were distributed lognormally. Therefore, emission data were transformed to their respective logarithms for all statistical analyses. Means of duplicate samples collected at each light and temperature combination for each plant were used to estimate the monoterpene emissions.

Data graphs for each plant showed that log monoterpene increased linearly with temperatures. Since a series of monoterpene measurements were made on a given plant while temperature was varied, all data points collected from the same plant were correlated violating the assumption of independent observations for regression analysis. Consequently, estimation of monoterpene emissions as a function of temperature could not be done simply by fitting a common regression line to the log data from all plants. Instead, a separate regression line was fit to each plant. Since monoterpenes were measured at the same temperature levels for all plants, the averages of the intercepts and slopes of the individual plant regression lines were optimum estimates (maximum likelihood estimates) of the intercept and slope of the population regression line (Graybill, 1976). By computing the variance and covariance of these slopes, and intercepts it was also possible to fit a confidence band about the estimated population response curve. Since the individual monoterpenes were also found to respond in a log-linear manner to temperature, for each, a regression line together with confidence bands was fit in the same manner as for the sum of monoterpenes. To determine if monoterpene production depended on light, first individual regression lines were fit to each plant which had

been exposed to varying light. The population intercepts and slopes were estimated as described above and a t-test was performed to test the hypothesis that the population slope equaled zero.

RESULTS. Five monoterpenes were found in the gas phase surrounding slash pine foliage in sufficient quantity to measure reliably (Figure 3). A sixth, camphene, was detected at a level too low for reliable measurement. Selected physical properties of these monoterpenes are listed in Table 1.

To determine the influence of light on monoterpene emission rates, light intensity was varied from 0 to 800 $\mu E/m^2/sec$ at each of four temperatures. A single regression line for log (sum of monoterpenes) vs light was fit to the data for each plant (Table 2). The slope parameter for each line was small and half had negative slopes. The average slope for all lines, the estimate of the population slope, was only -4.7×10^{-5}, indicating that monoterpene production changed less than 4% as light was increased from 0 to 800 $\mu E/m^2/sec$. According to a t-test, the regression of monoterpene emissions on light was not significant (slope not significantly different from zero), confirming that the emission rate of the sum of the monoterpenes did not depend on light intensity. Similar results were also obtained for the individual monoterpenes. In subsequent temperature studies, response curves developed at different light intensities were combined.

The influence of increasing temperature on monoterpene emission rates was determined by varying temperature from 20 to 46° C in the dark or at fixed light levels. Data for the sum of the five monoterpenes and individual monoterpenes are shown in Figure 4a-f. The sum of monoterpenes and each individual monoterpene was log-linearly related to temperature, even though there were large differences in the magnitudes of each component emitted. This log-

linear relationship means emissions increased exponentially with temperature. The percent variation (R^2) explained by the log-linear model was greater than 0.80 for all components except for limonene (0.71). At 35° C, α and β-pinene were emitted in the largest quantities (4.46 and 3.44 μg C/g dry wt/hr) while limonene, myrcene and β-phellandrene were only minor contributors to the total emissions: 0.70 μg C/g dry wt/hr for the sum of the three components. The slope parameters (Figure 4a-f) were approximately equal indicating that the proportion of each component was approximately constant over the temperature range studied.

To illustrate plant variability (Table 3), the individual slopes and the average monoterpene emission rates were determined for each of the 14 individual plants used to develop the temperature response curves. The slopes ranged from 0.011 to 0.053 with a mean of 0.032 while average emissions (at 35°C) range from 3.74 to 35.10 with a mean of 9.38 μg C/g dry wt/hr. The average monoterpene emissions were more variable than the slope parameters.

DISCUSSION. The qualitative composition of the monoterpenes in the vapor phase surrounding the slash pine foliage was similar to that reported in cortical oleoresins (Squillace, 1971). Hanover (1972) has shown that the monoterpene composition of the cortex oleoresins and the foliage of pine was quite similar. He also reported that foliar and vapor phase monoterpene compositions were qualitatively similar. However, the % vapor phase concentration of monoterpenes with low boiling points was frequently higher than the % foliar concentration. (Hanover, 1972). The monoterpenes emitted in highest quantity from slash pine were those with the lowest boiling points (Table 1).

Dement et al. (1975) reported that camphor volatilization rate depended

on vapor pressure. Our monoterpene emission rates increased in a log-linear manner with temperature (Figure 4). This log-linear relationship for emissions is expected since monoterpene vapor pressures are also log-linearly related to temperature (Jordan, 1954). This functional relationship, Log(monoterpene) = a + b (temperature), means that emissions increase exponentially with temperature. Specifically, it indicates that the emission rate of increase of each monoterpene with temperature will be simply porportional to the amount of monoterpene currently present. Hence, "b" in the above equation is the constant of proportionality, which means that at any instant, the rate of increase per degree of temperature, will be (b x 100)% of the current amount of monoterpene "b" is sometimes referred to as the relative rate of change.

The slope of the vapor pressure versus temperature curves is essentially equal for the monoterpenes listed in Table 1 and indicates a relative rate of increase in vapor pressure/°C of 2.4% (Jordan, 1954). This figure is similar to the average 3.2% relative rate of increase in monoterpene emission rates (Figure 4) indicating that vapor pressure was a significant factor in controlling monoterpene emissions. This suggests that only monoterpenes with appreciable vapor pressures at ambient temperatures will occur in significant concentrations in the atmosphere.

The increase in monoterpene emissions with temperature (Figure 4) is similar to previous results with the other species (Rasmussen, 1972; Tyson et al., 1974; Arnts et al., 1978; Kamiyama et al., 1978). When data from these studies (Rasmussen, 1972; Arnts et al., 1978; Kamiyama et al., 1978) were recalculated using the log-linear model, α-pinene emissions were found to increase at a relative rate of 5.3% (average of three pine and one fir species) 3.9% for loblolly pine and 3.5% for crytomeria. Our data (Figure 2)

indicated that α-pinene emissions would increase at a relative rate of 2.9% per degree.

The monoterpene emissions from slash pine were similar in the dark and at various light intensities. This response was similar to reports for other species (Rasmussen, 1972; Tyson et al., 1974). The lack of light influence on monoterpene emissions is in contrast to the light dependent emissions of the hemiterpene, isoprene, (Rasmussen and Jones, 1973; Tingey et al., 1978).

The environmental conditions for an average of summer days in Tampa, Florida, and the estimated monoterpene emission rates as a function of temperature (Figure 4) were used. to model typical diurnal monoterpene emission patterns. Light was assumed to have no direct effect on monoterpene emission rates (Table 1). Climatic summaries (e.g. Visher, 1954; NOAA, 1974) indicated an average daily solar radiation of approximately 500 Langleys, and average maximum and minimum air temperatures of 32 and 20° C, respectively during the summer. The average hourly air temperature was inferred from the observed average maximum and minimum temperatures and what is known about typical diurnal temperature cycles. Needle temperature was assumed to equal air temperature (Gates, 1962).

Estimated hourly emission rates of monoterpenes from slash pine in the vicinity of Tampa, Florida, are shown in Figure 5. The predicted maximum emission rates occurred shortly after mid-day and decreased to a minimum shortly before sunrise. Much of the daily monoterpene emissions occurred during mid-day and early evening. Mono-terpene emissions and reported in Figure 4 and Figure 5 estimated from the model were similar to the emission rates measured on slash pine in the Tampa, Florida area (P.R. Zimmerman, personal communication). The α-pinene emission rates from our slash pine and

loblolly pine (Arndts et al., 1978) were similar when expressed in the same units.

The equations relating log (monoterpene) to temperature can be used to compare emission rates measured in the field at different temperatures. Under the assumption that emission rates may differ in mean level but show the same relative rate of increase with temperature from site to site. The adjustment of emission rates to a standard temperature (i.e. 30°C) is straightforward. One simply multiplies the observed emission taken at same temperature "T" in the field by the ratio of the emission predicted at 30°C to that predicted at temperatures T. This ratio has the form $[e^{a + bX30}] / [e^{a + bXT}]$ - for the sum of monoterpenes it would be $[e^{-0.144 + 0.0317X30}] / [e^{-0.144 + 0.0317XT}]$.

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Table 1. THE MAJOR MONOTERPENES MEASURED IN THE GAS PHASE SURROUNDING SLASH PINE FOLIAGE

Compound	Boiling Point (°C)	Vapor Pressure[1/] (Torr)	Average[2/] Emission
α-Pinene	156	8.8	4.46
β-Pinene	164	5.9	3.44
Myrcene	167	3.4	0.32
Limonene	178	3.3	0.16
β-Phellandrene	171	2.5	0.22

1/ The vapor pressures listed were determined at 35°C and based on data of Jordan (1954). The vapor pressure of β-Phellandrene was assumed to equal α-Phellandrene

2/ Average monoterpene emission (μg C/g dry wt/hr) at 35°C

Table 2. EFFECT OF INCREASING LIGHT INTENSITY ON MONOTERPENE EMISSION RATES AT SEVERAL TEMPERATURES.

Temperature °C		Slope[1] b X 10^3
29		0.438
		-0.321
		-0.143
35		-0.373
		-1.680
		-0.781
40		-0.217
		1.224
		0.191
46		0.807
		0.291
		0.017
	$\bar{x}$[2]	-0.047
	σ	0.751

1/ The slopes are for the regression log (sum of monoterpene) on light, with each slope computed from data of a single plant.

2/ This mean is the best estimate of the regression slope for the population of plants. The ratio of $\bar{x}$ to σ yields a t-value to test if emissions are light dependent.

Table 3. PLANT VARIABILITY IN MEAN MONOTERPENE EMISSIONS AND RATE OF INCREASE OF EMISSIONS AS A FUNCTION OF TEMPERATURE[1/].

Plant	Average Monoterpene Emission[2/] µg C/g dry wt/hr	Slope[3/]
1	6.26	0.039
2	26.38	0.032
3	9.84	0.024
4	3.74	0.025
5	9.38	0.053
6	6.63	0.046
7	9.31	0.033
8	7.29	0.013
9	5.50	0.015
10	6.67	0.039
11	10.82	0.011
12	35.10	0.040
13	7.86	0.019
14	14.58	0.052
$\bar{x}$[4/]	9.38	0.032

1/ Data are based on the sum of the monoterpene.

2/ Average monoterpene emissions were determined at 35°C which was the mean of the temperature range used in calculating the linear regressions.

3/ Slope multiplied by 100 equals relative % increase in monoterpene per degree temperature.

4/ For average emissions, $\bar{x}$, is a geometric mean; for the slopes, $\bar{x}$, is an arithmetic mean.

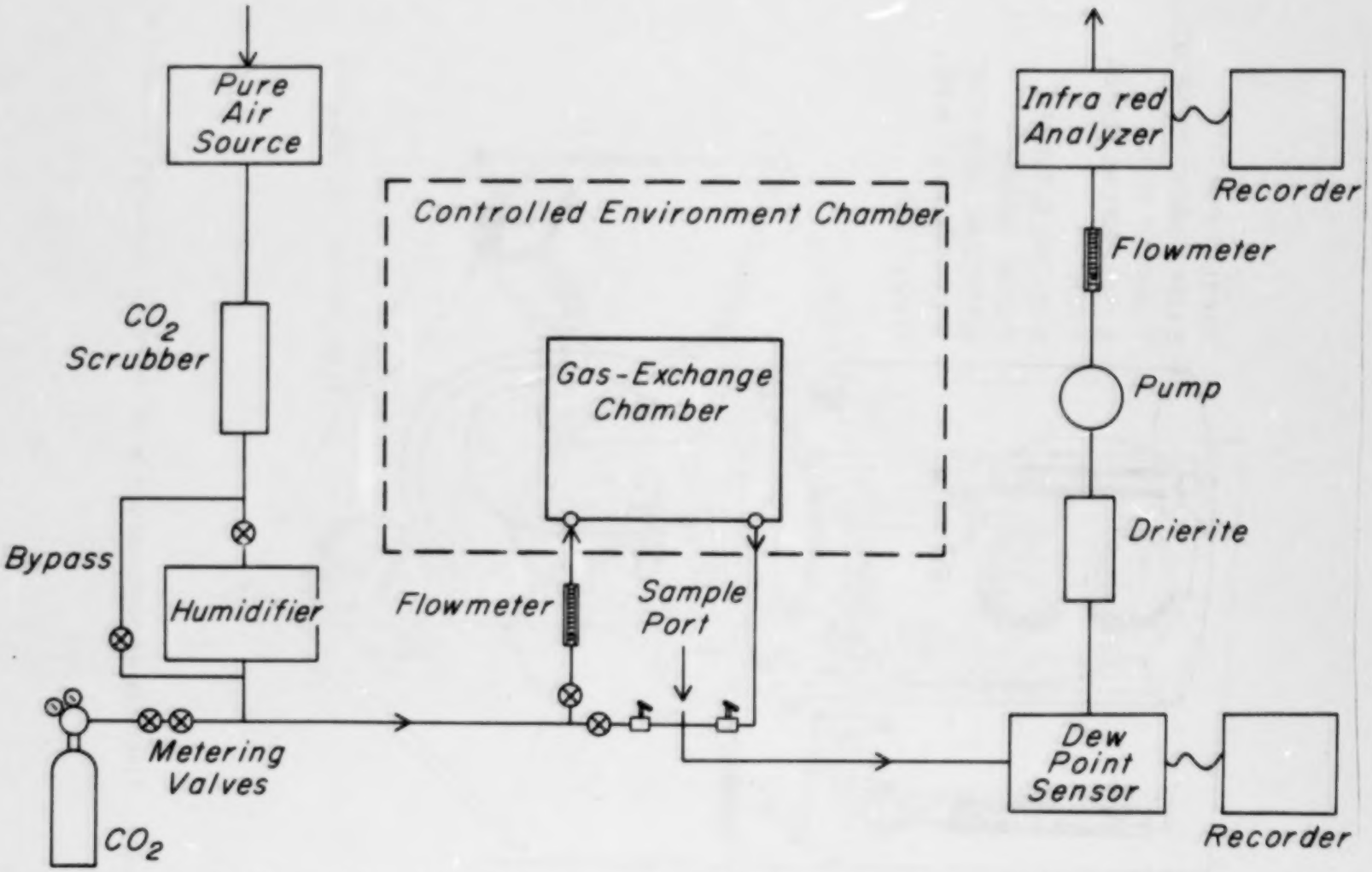

Figure 1. Air flow pattern through the gas-exchange chamber and arrangement of instrumentation.

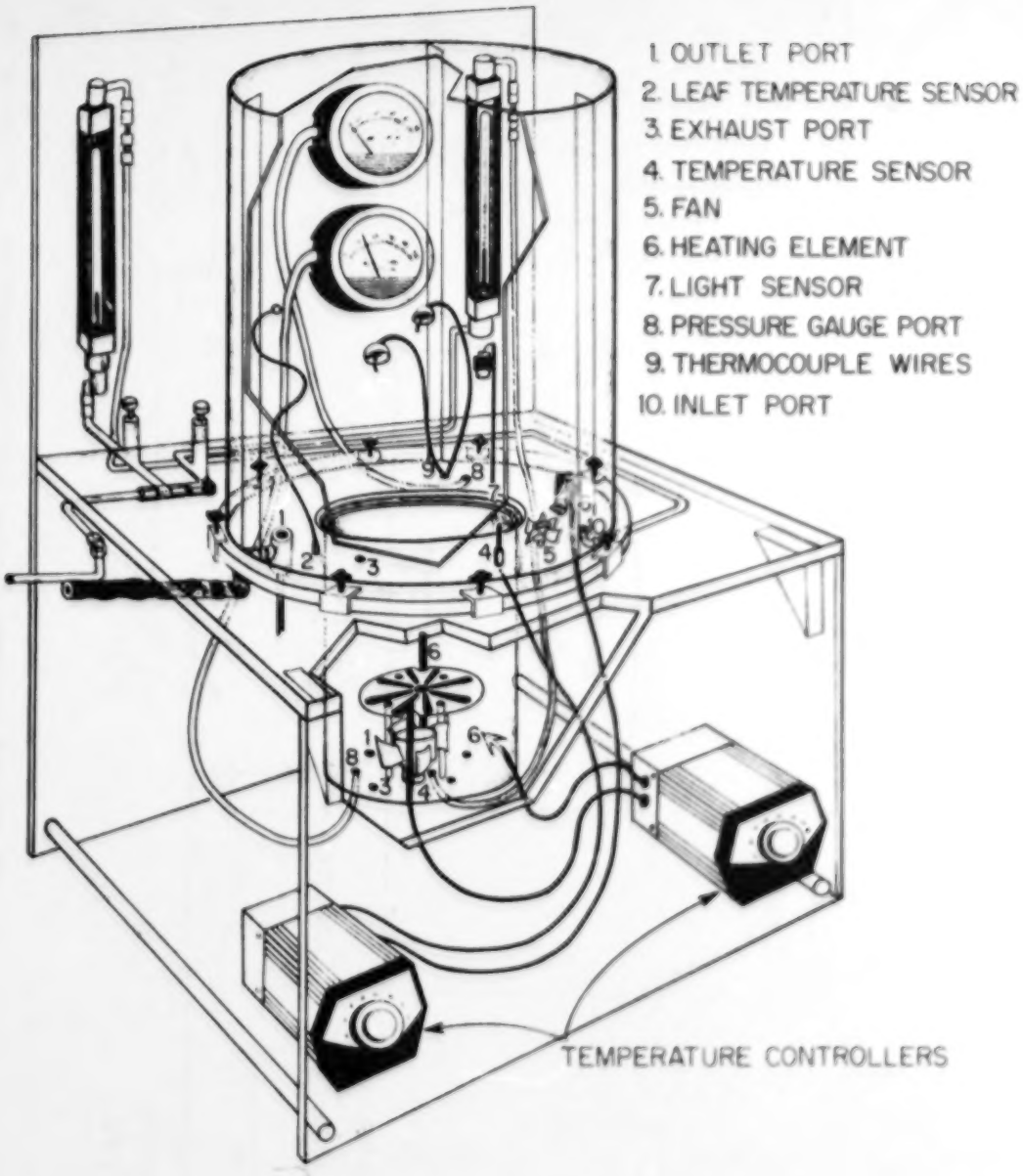

Figure 2. Plant gas-exchange chamber with the major components illustrated.

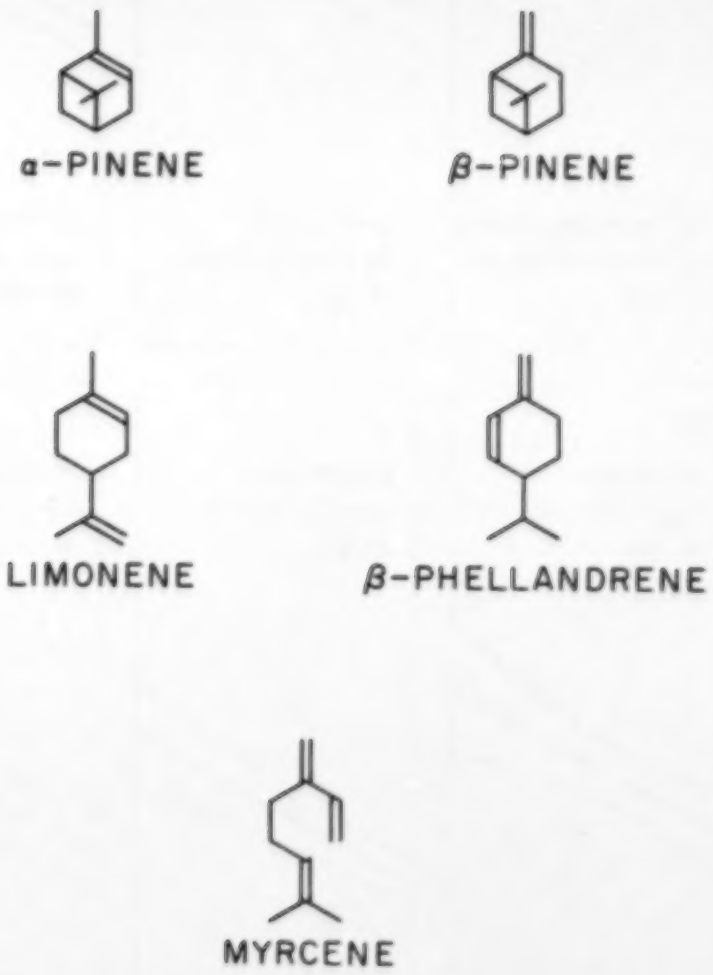

Figure 3. The structure of monoterpene detected in the vapor phase surrounding slash pine trees.

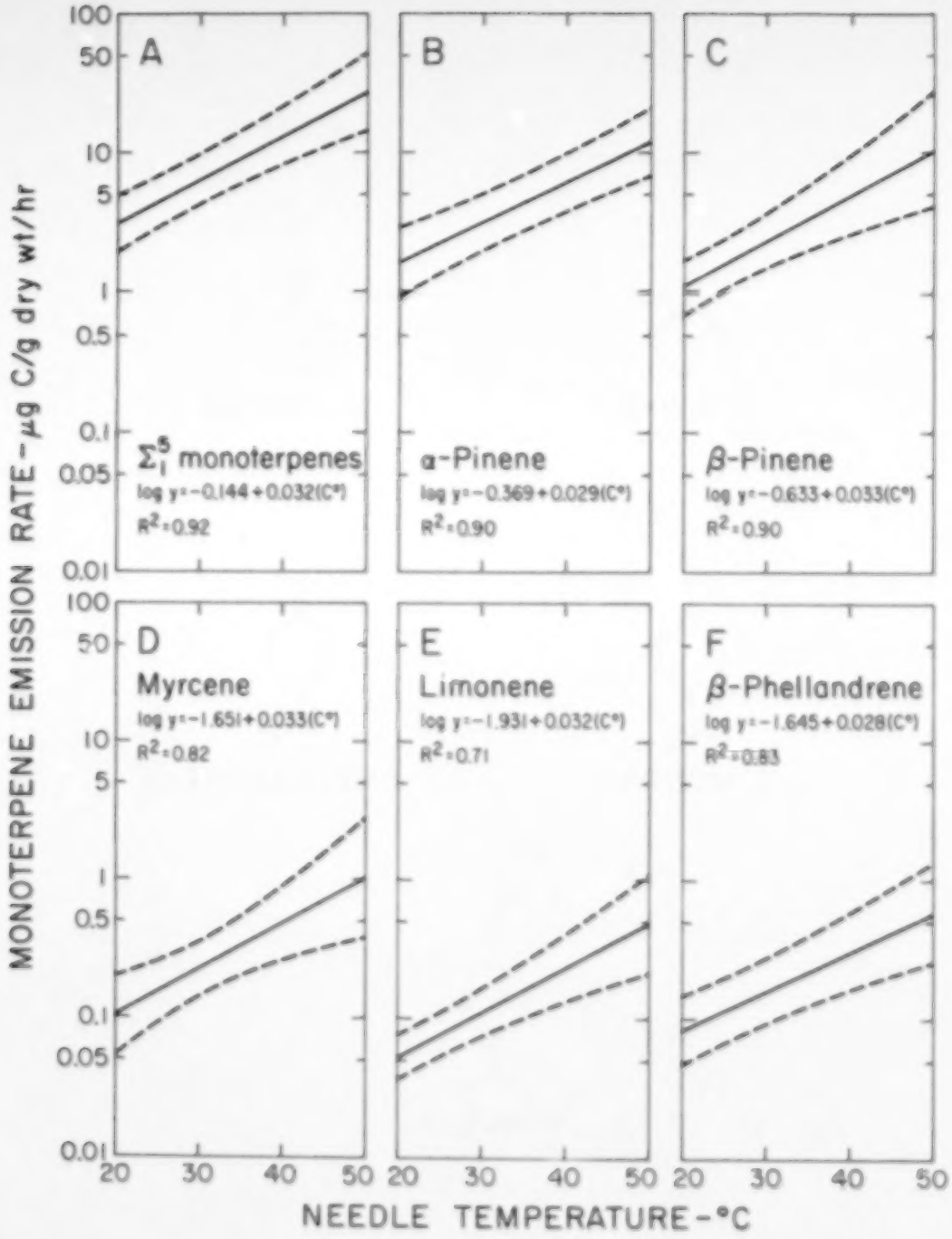

Figure 4. The influence of varying temperature on monoterpene emission rates in slash pine. Data from 14 plants were used to develop the temperature response curve.

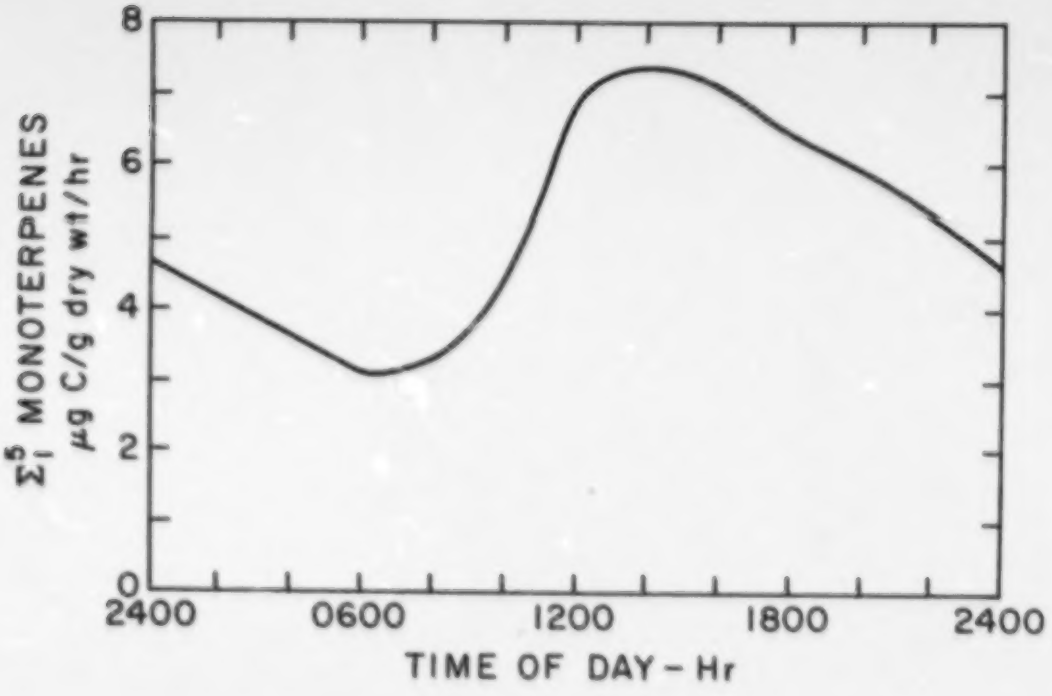

Figure 5. Estimated diurnal monoterpene emissions for slash pine in Tampa, Florida, for an average of summer days. Needle temperature was assumed to equal air temperature.